applying additional measures to those with known exposure to implicated batches.

27. This specific haemophilia patient had received such large quantities of Factor VIII – almost 400,000 units, the majority since 1980)] - that on these calculations, the cumulative risk from the "non-implicated" batches may well have exceeded that from the smaller number of "implicated" ones. This can be illustrated by considering the expected number of ID_{50} received via each route. This is illustrated in the second part of Annex A. In summary:

- If the two "implicated" pools contained 3 infected donations, this route would have exposed the patient to a total dose of 0.6 ID_{50}.
- If the other "non-implicated" pools each contained 2 infected donations, this route would have exposed the patient to an expected total of 24 ID_{50}.

28. Simple application of the linear dose-response model would then suggest that whereas Factor VIII from the two "implicated" pools would have contained a dose liable to transmit infection with a probability of 0.3, the large number of units sourced from "non-implicated" pools would have contained more than enough infectivity to transmit. Crudely, this suggests that the "non-implicated" pools represent the more probable source of infection, by a factor of just over 3.[4]

29. This last calculation is reflected in Table 2 below, for prevalence scenarios of both 1 in 10,000 and 1 in 4,000. However, we stress that this is very simplistic. It rests on accepting the linear model uncritically, and assuming that doses received on successive occasions can simply be added together in calculating an overall risk of infection. Nevertheless, the comparison between "implicated" and "non-implicated" routes is instructive, in showing how the sheer number of exposures may come to dominate the presence of a known infection.

Table 2: Relative probabilities of potential infection routes (including "non implicated plasma" products)

Prevalence, p	1 in 4,000		1 in 10,000	
Transmission probability, t1	0.5	1	0.5	1
Probability implicated plasma products	38%	38%	24%	24%
Probability of each of the 14 component donors	<0.03%	<0.03%	<0.02%	<0.02%
Probability primary	<0.03%	<0.03%	<0.02%	<0.02%
Probability non-implicated plasma products	61%	61%	76%	76%

Note: these are illustrative calculations only. All figures are rounded to the nearest %, or (for small probabilities) indicate an upper bound.

[4] Note that the differential between *infectious doses* is much greater, but the practical effect is limited by infection being regarded as certain once the dose reaches 2 ID_{50}. As seen in following paragraphs, the risk differential between routes is therefore more pronounced in lower-infectivity scenarios.

30. As can be seen, the previous conclusion about the low implied risk to each of the 14 component (red cell) donors still applies, with even greater force. However, these results also highlight something of a paradox. Combined with the infectivity scenario taken from the DNV assessment, the pool size / prevalence calculations suggest that many recipients of plasma products would have received very high infectious doses, *whether or not* they had received any "implicated" units with known linkage to an infected donor. This opens the question of why no clinical vCJD cases have been seen in the population of haemophilia / blood disorder patients designated as "at risk" because of their exposure to UK sourced blood products.[5] It might therefore be argued that the infectivity assumptions applied to plasma products are overly pessimistic.

31. Although this question is impossible to answer definitely, and in any case raises issues beyond the scope of this paper, it is appropriate to check that the conclusions we have already suggested about relative likelihoods would not be overturned were we to assume lower levels of infectivity in plasma derivatives. The DNV report itself suggests two possible methods for calculating the infectivity present in each plasma derivative, using different assumption about the effect of the various manufacturing steps. In line with the generally precautionary approach adopted by CJD Incidents Panel, the calculations so far use figures based on the more pessimistic of these. The less pessimistic alternative suggested by DNV (using the "highest single clearance factor" in the manufacturing process) leads to an infectivity estimate for Factor VIII that is lower by a factor of 4. However, it should also be noted that risk assessments carried out elsewhere take the clearance factors achieved at different stages to be at least partly additive, which would lead to much smaller infective loads.

32. In fact, reducing the assumed infectivity *increases* the relative chance of infection via "non-implicated" as compared to "implicated" plasma. For example, suppose the presumed infectivity in all the Factor VIII received was reduced by a factor of 100 (2 logs). Modifying the calculations in paragraph 27, this patient would then have received an expected:

- 0.006 ID_{50} from the two "implicated" pools (representing a transmission risk of 0.003)
- 0.24 ID_{50} from all the other "non-implicated" pools (representing an infection risk of 0.12).

33. Albeit with the same caveats as before about using the linear model to quantify the cumulative risks from successive doses, this suggests that the latter risk would outweigh the former by a factor of 40. Table 3 shows how the previous results for this patient would change, under this revised infectivity scenario. As can be

[5] *Possible* explanations include the following: that prevalence of infection amongst donors is much lower than in the scenarios considered here; that much more infectivity is removed during processing of plasma products than suggested by the DNV analysis; and/or there is a threshold dose-response effect and most recipients fall below this. Genotype effects may also be relevant (in providing resistance to infection or extending the time to clinical disease), but one would expect a substantial proportion of this group to be MM homozygotes – the most susceptible genotype.

seen, the previous conclusions still hold, in particular regarding the small implied risk to each of the 14 red cell donors.

Table 3: Relative probabilities of potential infection routes (including "non implicated plasma" products and using lower infectivity estimates for plasma products)

Prevalence, p	1 in 4,000		1 in 10,000	
Transmission probability, t1	0.5	1	0.5	1
Probability implicated plasma products	2%	2%	3%	3%
Probability of each of the 14 component donors	<0.05%	<0.09%	<0.05%	<0.09%
Probability primary	<0.09%	<0.09%	<0.09%	<0.09%
Probability non-implicated plasma products	97%	97%	97%	96%

Note: these are illustrative calculations only. All figures are rounded to the nearest %, or (for small probabilities) indicate an upper bound.

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Annex A: Application of DNV Risk Calculation to Factor VIII Units

(a) Implicated Donations

Key points: FHB4547

- There was one implicated (presumed infective) donation in a start pool of 26,303 donations (pool size supplied by Professor Frank Hill via email)
- Factor VIII is derived from cryoprecipitate, which has an estimated infectivity of 60 ID_{50}s / donation of infected whole blood according to the DNV model
- 70.45kg of cryoprecipitate was made from the start pool, of which 21.58kg was used in the FHB4547 batch
- This implies that (21.58kg / 70.45kg) of the 60 ID_{50}s made its way into the FHB4547 batch (18.38 ID_{50}s)
- 1,844 vials each of 500 units (iu) were made from the batch, which results in an estimate of 0.00997 ID_{50}s per vial or 1.99×10^{-5} ID50s per iu

Professor Frank Hill's report indicates that the index case received 8,025 units from this batch, giving an estimated 0.16 ID_{50} from the implicated donation.

Key points: FHC4237

- There was one implicated (presumed infective) donation in a pool of 21,330 donations (pool size again supplied by Professor Frank Hill)
- Factor VIII is derived from cryoprecipitate, which has an estimated infectivity of 60 ID_{50} / donation of whole blood
- 67.6kg of cryoprecipitate was made from the start pool, of which all was used in the FHC4237 batch
- This implies that the full dose of 60 ID_{50} made its way into the FHC4237 batch
- 5,074 vials each of 250 iu were made from the batch, resulting in an estimate of 0.0118 ID_{50} per vial or 4.73×10^{-5} ID_{50} per iu

Professor Frank Hill's report indicates that the index case received 1,000 units from this batch, giving an estimated dose of 0.05 ID_{50}.

Conclusion

In total, these calculations suggest that index case would have received an estimated 0.21 ID_{50} from the "implicated" donor. Using a linear dose-response model (where 1 ID_{50} translates into a transmission probability of 0.5 and 2 ID_{50} or more translates into transmission probability of 1) this represents a transmission probability of 0.104 or 10.4%.

(b) Non-implicated Donations

In addition to the implicated donations, we have also to consider the possibility of other donors contributing to a pool being infective. With pool sizes of the order of 20,000 donations, each pool will be likely to contain contributions from one or more infected donors by chance, unless p is very small. For implicated pools, these will be *in addition to* the "known" implicated donor.

With a prevalence of 1 in 10,000, one might therefore expect the two implicated pools to contain two *further* infected donations, taking the total from 1 to 3 per pool.

This would make the infective dose received via the implicated units three times that calculated above, i.e. a total of roughly 0.6 ID_{50}, yielding a transmission probability of 0.3.

This patient also received approximately 391,000 iu of UK-sourced Factor VIII plasma treatment *not* known to be associated with any infected donor. In round figures, this can be visualised in terms of 20 exposures to pools of 20,000 donors, each typically containing 2 donations from infected donors. The exact infective dose passed on to the patient will vary from batch to batch. However, the two examples given in part (a) suggest an eventual dose of 2-5 x 10^{-5} ID_{50} per unit, per infected donor. For illustration, therefore, suppose that each unit exposed the recipient to 6 x 10^{-5} ID_{50}, 400,000 such units would therefore have exposed the recipient to 24 ID_{50}.

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ORIGINAL PAPER

Distribution of a bovine spongiform encephalopathy-derived agent over ion-exchange chromatography used in the preparation of concentrates of fibrinogen and factor VIII

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Background and Objectives The risk of haemophiliacs contracting variant Creutzfeldt-Jakob disease (vCJD) via treatment with factor VIII concentrates is not known. Therefore, in order to determine the extent to which the vCJD agent might be removed during the preparation of factor VIII concentrate, the partitioning of a bovine spongiform encephalopathy (BSE)-derived agent was measured over the main purification step used to prepare the Scottish National Blood Transfusion Service high-purity factor VIII concentrate (Liberate®).

Materials and Methods Murine-passaged BSE (strain 301V), in the form of a microsomal fraction prepared from infected brain, was used to 'spike' a solution of factor VIII of intermediate purity. The 'spiked' starting material was subjected to solvent-detergent treatment and then to anion-exchange chromatography with Toyopearl DEAE-650M. All fractions were tested for 301V infectivity using a murine bioassay, including the procedures used to clean the ion-exchange media after use.

Results BSE 301V infectivity was reduced by 2·9 $\log_{10}$ in the fibrinogen fraction and by 2·7 $\log_{10}$ in the factor VIII fraction. Over 99% of the added 301V infectivity remained bound to the ion-exchange column after elution of factor VIII. A large quantity of infectivity was subsequently removed by washing the ion-exchange media with 2 M NaCl. No further BSE 301V infectivity was detected in column eluates after treatment with 0·1 M NaOH or a second wash with 2 M NaCl.

Conclusions Results using a BSE-derived agent suggest that vCJD infectivity would be substantially removed by the ion-exchange process used in the preparation of fibrinogen and factor VIII concentrate. Although 301V infectivity remained bound to the ion-exchange matrix following elution of factor VIII, this appeared to be eliminated by the procedure used for cleaning the ion-exchange media after each use.

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Key words: Creutzfeldt–Jakob disease, chromatography, factor VIII, fibrinogen.

Introduction

Variant Creutzfeldt–Jakob disease (vCJD) is an incurable, fatal, neurodegenerative disorder of transmissible spongiform encephalopathy (TSE), of which there have been 150 confirmed or probable cases diagnosed since the condition was first reported [1]; 143 of these 150 subjects were resident in the UK at some time. Evidence that vCJD is caused by the TSE agent responsible for bovine spongiform encephalopathy (BSE) in cattle is convincing [2], with dietary exposure being the most probable route of transmission [3]. BSE originated in the UK [4] and has now been detected in cattle in 25 different countries, although 98% of all cases found

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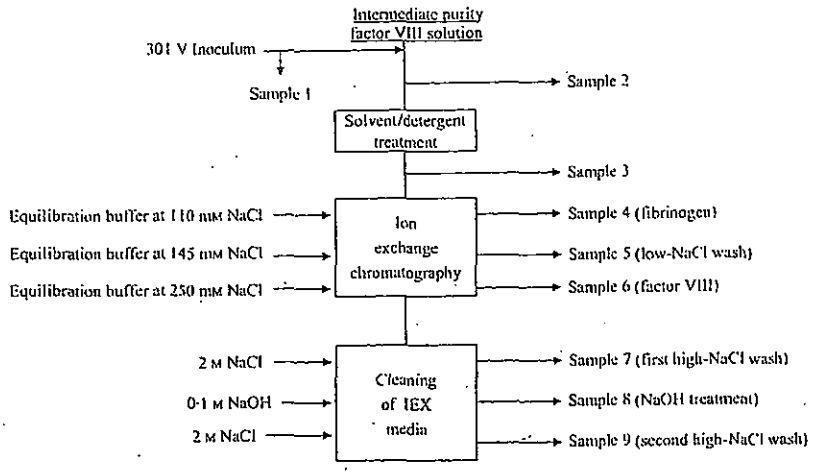

Fig. 1 Flow diagram of the processes over which partitioning of bovine spongiform encephalopathy (BSE) 301V infectivity was measured. IEX, ion-exchange chromatography.

of equilibration buffer, with the breakthrough (unadsorbed) fraction (139·8 ml) being collected (fibrinogen fraction). Forty-one millilitres of equilibration buffer, containing 145 mM NaCl, was then applied and the resultant wash fraction collected (low-NaCl wash). This was followed by 26 ml of equilibration buffer containing 250 mM NaCl, at a flow-rate of 48 ml/h, to elute factor VIII (factor VIII fraction).

Cleaning of the ion-exchange gel

Following collection of the factor VIII eluate, the chromatography bed was cleaned *in situ* by washing with 2 M NaCl, followed by 0·1 M NaOH and then again with 2 M NaCl. First, 25 ml of 2 M NaCl was applied to the column and the eluate (15·2 ml) was collected from the beginning of the 'salt front' (first high-NaCl wash). Subsequently, 0·1 M NaOH (70 ml) was applied to the column and an eluate (39 ml) was collected when the pH increased from 6·3 to > 12 (NaOH wash). When the application of 0·1 M NaOH was complete, the column was allowed to soak in NaOH for 1 h and then subjected to a second wash with 2 M NaCl (42 ml). An eluate volume of 8·1 ml was collected to capture the protein-containing fraction observed at this stage (second high-NaCl wash).

Determination of protein elution during the ion-exchange process

Throughout the ion-exchange procedure, the output from the column was monitored continuously by inline measurement of the solution optical density at a wavelength of 280 nm (OD_{280}) to detect total protein being eluted (Fig. 2).

Scale-down of the ion-exchange process

The small-scale ion-exchange procedure used in this study was designed to give yields and purification for factor VIII

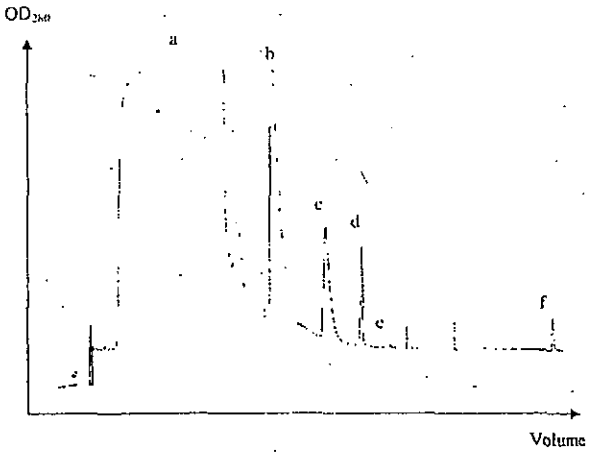

Fig. 2 Optical density of fractions eluted during ion-exchange chromatography of intermediate-purity factor VIII to which the bovine spongiform encephalopathy (BSE) 301V microsomal inoculum had been added. (a) Fibrinogen fraction (110 mM NaCl); (b) low-NaCl wash (145 mM NaCl); (c) factor VIII fraction (250 mM NaCl); (d) first high-NaCl wash (2 M NaCl); (e) NaOH wash (0·1 M NaOH); (f) second high-NaCl wash (2 M NaCl).

and fibrinogen equivalent to the full-scale process. Although the degree of scale-down was ≈ 1300-fold, all materials and surfaces were the same as in routine manufacture, except that chromatography eluates were collected into polypropylene containers rather than stainless steel vessels. The OD_{280} profile obtained in the presence of added 301V (Fig. 2) was the same as that obtained in the absence of 301V, both in the small-scale model and in the routine full-scale chromatography process, demonstrating the accuracy of down-scaling achieved.

Determination of BSE 301V infectivity

The BSE 301V infectivity of samples from the ion-exchange process was determined by bioassay. Samples for assay were diluted in saline and injected intracerebrally (20 µl) into

